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Potential antiviral drug resistance mutations in patients with treatment-naive chronic hepatitis B

Antiviral drug resistance mutations at patients with chronic hepatitis B

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Abstract

Aim: The prevalence and clinical effects of mutations in the reverse transcriptase (RT) region of the Hepatitis B Virus (HBV) before antiviral treatment in patients with chronic Hepatitis B (CHB) are not clear. In this study, the aim was to investigate the prevalence, characteristics, antiviral drug resistance, and clinical effects of mutations in the RT region of the HBV polymerase gene in patients with treatment-naive CHB.

Material and Methods: The study included 102 treatment-naïve patients who underwent liver biopsy due to chronic hepatitis B disease. The gene region encoding HBV RT enzyme was amplified by PCR and Sanger sequencing was performed.

Results: The genotype of all patients with CHB was defined as HBV/D. In the study, among the 102 included patients, 82 (80.39%) had a total of 35 types of mutations associated with the 42 previously identified Nucleos(t)ide analogs (NAs) resistance (NAr) mutation positions in the HBV RT region. In a total of 5 patients (4.90%), primary and secondary drug resistance were detected. In this study, 22 new mutations were identified in 13 out of 31 RT positions associated with NAr. No statistically significant differences were observed in terms of liver enzymes and histologies, as well as virological features including HBV DNA levels, between patients with detected NAr mutations and those without.

Discussion: Although the prevalence of amino acid (AA) variations in the HBV RT region is high in patients with HBV/D genotype, primary and secondary antiviral resistance associated with NAs is rare. Effects of NAr mutations on the progression of HBV-related diseases are also quite limited.

Keywords

Hepatitis B Virus, Chronic Hepatitis B, Treatment-Naive, Mutations, Polymerase Reverse Transcriptase

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Introduction

Chronic hepatitis B virus (HBV) infections continue to be a significant clinical problem worldwide. [1]. Currently, there is no effective treatment for the estimated 296 million individuals worldwide who are carriers of chronic HBV. It is estimated that approximately 820,000 people die from HBV-related liver diseases each year [2].

HBV replication occurs through the reverse transcription of viral pregenomic RNA. In this respect, HBV Polymerase/reverse transcriptase (Pol/RT) is a critically important enzyme for viral replication and serves as an antiviral target for the inhibition of HBV biosynthesis. [3]. Today, antiviral treatment strategies designed to achieve prolonged suppression of viral replication by targeting HBV RT include clinical use of nucleotide analogs (NAs) such as lamivudine (LMV), adefovir dipivoxil (ADV), telbivudine (LdT), entecavir (ETV), and tenofovir (TDF) for the treatment of patients with chronic hepatitis B (CHB) [4].

However, long-term treatments and mutations arising from the lack of proofreading ability of the HBV RT enzyme can lead to the development of antiviral drug resistance. Antiviral drug resistance caused by RT mutations can limit the clinical efficacy of treatment, leading to treatment failure and an increase in the progression of the disease. Moreover, mutations arising from the partially overlapping genomic structure of HBV's surface antigen (HBsAg), encoded by the HBV surface (S) gene, with the RT can affect the antigenicity, immune recognition, virulence, and replication capacity of HBV [5].

In patients with CHB, 42 potential nucleotide analog resistance (NAr) mutation positions have been identified in the HBV RT region prior to treatment. However, the prevalence of preexisting mutations in the HBV polymerase gene's RT region and their impact on antiviral treatment in treatment-naive patients with CHB is not yet clear [6, 7].

Therefore, the objective of this study is to determine the prevalence of preexisting mutations in the HBV RT region in treatment-naive patients with chronic hepatitis B (CHB) and to examine the potential and clinical effects of these mutations on antiviral treatment.

Material and Methods

This study was supported by the Scientific Research Projects Coordination Unit of Istanbul University with the project number TSA-2023-39107. All patients included in this study gave informed consent for participation.

Patients:

This study included 102 patients followed by the Gastroenterohepatology clinic of Istanbul University Istanbul Faculty of Medicine due to CHB disease. Liver biopsy procedures were performed at the Department of Internal Medicine, Division of Gastroenterohepatology, and the Interventional Radiology Unit of the Department of Radiology at Istanbul Faculty of Medicine.

Amplification of HBV Pol/RT gene:

Total genomic DNA samples, isolated from liver biopsy specimens of patients using a commercial kit (QIAamp DNA Mini kit, Qiagen GmbH, Hilden, Germany), were amplified by PCR method using HBV Pol/RT gene-specific primers (Table 1). To amplify the target region, a PCR setup (25 µl) was constructed, containing 12.5 µl 2X PCR master mix (HS Prime Taq Premix; GeNet Bio) and external primers (200ng/µl) for amplification of 5µl DNA extract. PCR conditions were as follows: 95°C for 10 min; 35 cycles of 95°C for 30 seconds, 55-60°C for 1 min and 72°C for 1 min; then 72°C for 10 min.

Sequencing of HBV Pol/RT gene:

PCR products detected to have bands of the desired length were purified for sequencing. The sequencing of the purified amplification products was carried out using amplification primers in a bidirectional manner through a genetic analyzer (Beckman Coulter CEQ 8000).

The nucleotide sequences of the HBV Pol/RT gene obtained after the sequencing process were edited using the Chromas program. The confirmation and bioinformatic analyses of the edited sequences were performed on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). In determining the HBV genotypes, the NCBI website containing HBV reference sequences (www.ncbi.nlm.nih.gov/projects/genotyping) was used.

To identify mutations in the HBV Pol/RT gene, the Geno2pheno tool of the Max Planck Institute Informatik (https://hbv.geno2pheno.org/index.php) and the HIV-grade HBV Drug Resistance Interpretation program (http://www.hiv-grade.de/hbv grade/deployed/grade) were used.

The demographic, clinical, pathological, and laboratory data of the patients were obtained from patient files or, when necessary, from the hospital's electronic records.

Statistical analysis

Statistical analyses were conducted using the SPSS software (version 25.0, SPSS Inc., Chicago, IL). The results were presented as means and standard deviations. The Chi-square test and/or Fisher test were used for categorical variables, and Mann-Whitney U and One-Way ANOVA tests were used to compare non-categorical variables. $P < 0.05$ was considered statistically significant. This study was approved by the Ethics Committee of Istanbul Medical School Ethics Committee at Istanbul University (Date: 2018.06.12, No: 895).

Results

Patients' characteristics

The data regarding the demographic, clinical, histological, and laboratory results of the patients included in this study are summarized in Table 2. The study included 51 female and 51 male patients with chronic hepatitis B (CHB), with an average

Table 1. PCR amplification and sequencing primers

Primer	Sequence (5'-3')	Polarity	Target	Position
HBVRT1	GCCTCATTMTGTGGGTCACCATA	Sense	Pol/RT	2801-2824
HBVRT2	CCTGCTGGTGGCTCCAGTTCA	Sense	Pol/RT	56–76
HBVRT3	TGCTGCTATGCCTCATCTTC	Sense	Pol/RT	414-433
HBVRT4	GGACGGAAATTGCACCTGTAT	Sense	Pol/RT	585-605
HBVRT5	TGGCTCAGTTTACTAGTGCCA	Sense	Pol/RT	670-690
HBVRT6	GAAGATGAGGCATAGCAGCAGG	Anti-sense	Pol/RT	433-412
HBVRT7	AATGGCACTAGTAAACTGAG	Anti-sense	Pol/RT	690-671
HBVRT8	CGTTGACAGACTTTCCAATCAAT	Anti-sense	Pol/RT	995-973
HBVRT9	GGTTGCGTCAGCAAACACTTG	Anti-sense	Pol/RT	1197-1177
HBVRT10	TTCCGCAGTATGGATCGGCAG	Anti-sense	Pol/RT	1278-1258

age of 41.7±13. The mean necroinflammatory activity scores and fibrosis stage levels of the patients with chronic hepatitis B included in the study were determined as 5 (0-17) and 2(0-6), respectively. The mean serum HBV DNA levels for patients with chronic hepatitis B (CHB) were Log10 5.31 (1.56 - >8.23).

Mutations on HBV Pol/RT gen of patients with CHB

The genotype of all isolated HBVs from patients with CHB was determined to be HBV D in 100% of cases.

Out of the 102 patients included in the study, at least one of the previously identified 42 NAr mutation positions in the RT region was detected in 82 (80.39%) individuals. In this study, 35 types of mutations were identified in 21 of 42 (50%) previously identified potential mutation positions for the RT region of the HBV polymerase gene (Tablo 3).

In this study, primary and secondary drug resistance were detected in a total of 5 patients (4.90%), with 4 patients (3.92%) showing resistance at the rtA194 position associated with TDF, and 1 patient (0.98%) at the rtV173 position (Tablo 3). No mutations associated with the previously identified primary and secondary drug resistance at 11 HBV RT positions were found in any of the patients, except for the two mentioned above.

In our study, 15 out of the previously identified 26 assumed NAr-associated mutation positions in the HBV RT region (57.7%) were detected. Nine out of these 15 RT positions were found to have 10 assumed mutations as described by Liu et al [6]. Additionally, in this study, 16 new mutations were identified at the 10 RT positions, different from the assumed mutations defined by Liu et al. The Y54H mutation, identified in 16 patients (15.68%), was the most common variation. (Tablo 3).

In this study, 5 out of the 6 previously identified pre-treatment RT mutation positions in the HBV RT region (83.3%) were detected. The pre-treatment mutation R242A, as defined by Liu and colleagues, was identified at only one RT position and in one patient in this study. Also, in this study, 7 new mutations were identified at 4 RT positions, different from the pre-treatment mutations defined by Liu et al, including N139T. Ultimately, in this study, 22 new mutations were identified in 13 of the 31 RT positions, (Tablo 3).

Thirteen out of the 21 amino acid positions where mutations were detected were genotype-associated polymorphic sites. In this study including treatment-naive patients, the amino acid (AA) positions rt38, rt53, rt54, rt124, rtH126, rt134, rt139, rt166, rt191, rt214, rt215, rt238, and rt256 in the HBV RT region were identified as the most polymorphic 13 (AA) positions. Among these, rtH124, detected in 41 patients (40.19%), was the most polymorphic amino acid position. The mutations detected in the functional domains (G, F, A, B, C, D, and E) and interdomains (F-A, A-B, B-C, C-D, and D-E) of patients in the HBV RT region, along with their prevalence, are showed in Table 4 in this study. Although mutations were detected at all sites, the most frequently mutated domain was identified as the A-B interdomain. The amino acid positions detected in this interdomain were R110, L115, N118, F122, H126, T128, S135, S137, N139, and Q149. H126R, T128I, and N139T were the most frequently observed variations in this domain. The C-D interdomain also contained the most variations with mutations at amino acid positions S213, V214, Q215, F221, and L229.

The average age of patients with identified NAr mutation included in this study was statistically significantly lower than that of patients in whom the mutation was not detected ($p<0.05$). No statistically significant differences were observed between patients with and without NAr mutations in terms of demographic information including gender, clinical laboratory data including AST, ALT, and AFP, liver histologies containing inflammation and fibrosis scores, and virological features including HBV DNA levels. (Tablo 2).

Discussion

This study aimed to investigate the prevalence, characteristics, and potential clinical implications of amino acid changes at the 42 positions associated with NAr within the HBV polymerase RT region in patients with CHB.

The 42 potential NAr mutations identified in the pre-treatment HBV RT region are classified into four categories: primary, secondary/compensatory, putative, and pretreatment mutations. Primary and secondary/compensatory resistance mutations are well-defined classical antiviral drug resistance mutations by in vivo and in vitro phenotypic experiments. Putative resistance mutations have their association with drug resistance defined, but these mutations have not yet been experimentally confirmed in vitro. In this regard, 26 putative RT mutation positions associated with NAr have been identified. Pretreatment mutations are as defined that the association

Table 2. Demographic, clinical laboratory, histopathological and virologic characteristics of all patients

Characteristic of Patients s	All Patients n=102	Patients with mutation NAr n=82	Patients without mutation NAr n=20	p
Mean age ± SD (years)	41.7±13	36.9±13	43.1±13	<0.05
Gender (M/F)	51/51	41/41	11/9	n.s.
Clinical laboratory				
ALT (U/L), Mean±SD	31 (14-339)	35 (16-171)	29.5 (14-339)	n.s
AST (U/L), Mean±SD	39.5 (13-634)	43.5 (15-106)	39 (13-634)	n.s
AFP (ng/ml), Mean±SD	3.19 (0.83-24.73)	2.355 (1.28-6.33)	3.29 (0.83-24.73)	n.s
Histology				
İnflammation (Grade) Mean±SD	5 (0-17)	4 (0-8)	5 (1-17)	n.s
Fibrozis (Stage), Mean±SD	2 (0-6)	2 (0-3)	2 (0-6)	n.s
Virologic factor				
HBV DNA(log 10IU/mL), (mean±SD)	5.31 (1.56 - >8.23)	5.53 (1.56 - >8.23)	5.30 (3.08 - >8.23)	n.s

Abbreviations: NAr: nucleos(t)ide analogue resistance, ALT; alanine aminotrasferase, AST; aspartate aminotrasferase, AFP; serum alpha-fetoprotein, n.s: not significant.

between with drug resistance has not been yet evaluated [6, 7]. (Tablo 3).

In this study, mutations were identified in 35 different types at 21 out of the previously defined 42 NAr positions in the RT region of the HBV polymerase gene in 82 patients (80.39%). The prevalence of pre-treatment RT mutations reported in treatment-naive patients can vary considerably. These data indicate the presence of a high prevalence of NAr-associated mutations in the Pol/RT region, as previously reported, in treatment-naive patients with HBV/D genotypes. [7, 8].

The prevalence of primary and secondary drug resistance in the pre-treatment HBV RT region is reported to be less than 5%, with the most frequently observed mutations being rtM204I/V and rtL180M, respectively [9]. In this study, the prevalence of primary and secondary drug resistance was found to be 3.92% and 0.98%, respectively, in total 4.90%, which is less than 5%. However, the most frequently observed mutations included TDF-associated rtA194T and LAM-associated rtV173L, different from rtM204I/V and rtL180M, respectively (Tablo 3). These data indicate that spontaneous antiviral resistance is quite rare in treatment-naive patients with HBV/D genotypes included in this study, and there is no significant limitation to the use of NAs in patients with CHB.

In our study, a total of 26 mutations were identified in 15

Table 3. Mutations associated with HBV reverse transcriptase

Mutation category	RT mutation typea	Relationship with antiviral	Mutations defined in this study (n)	Prevalence (%) (n:102)
Primaryb	I169T	ETV	A194T (4)	3.92
	A181T/V	LMV, LdT, ADV, TNF		
	T184A/C/F/G/I/L/M/S	ETV, LMV		
	A194T	ADV, TNF		
	S202C/G/I	ETV, LMV		
	M204I/V/S,	LMV, LdT, ADV, TNF, ETV		
	N236T	ADV, TNF		
	M250I/L/V	ETV		
Secondary/compensatory	L80I/V, V173L	LMV	V173L (1)	0.98
	L180M	LMV, ETV, LdT		
Putativec	S53N	LMV	N53D/H/E (3,1,1)	2.94, 0.98, 0.98
	T54N	ADV	Y54H/N//D (16,3,1)	15.68, 2.94, 0.98
	L82M	LMV		
	V84M, S85A	ADV		
	I91L	LMV	I91L (7)	6.86
	Y126C	ADV	H126R (8)	7.84
	T128I, T128N	LMV	T128I (5)	4.90
	N139D	LMV	N139T (2)	1.96
	W153Q	LMV		
	F166L	LMV	F166L (4)	3.92
			F166I (1)	0.98
	V191I	LMV, ADV	V191G (2)	1.96
	A200V, V207I	LMV		
	S213T	LMV, ETV	S213T (2)	1.96
	V214A	ADV	V214A (1)	0.98
			V214I (2)	1.96
			V214P (1)	0.98
	Q215P/S	LMV, ADV	Q215/H (3)	2.94, 0.98, 5.88
			Q215P (1)	
			Q215S (6)	
	L217R, E218D, F221Y	ADV	F221Y (8)	7.84
	L229G/V/W	LMV	L229V (1)	0.98
	I233V, P237H,	ADV	N238H (1)	0.98
	N238D/S/T, Y245H			
	S/C256G	LMV, ETV	C256S (12), C256G (2)	11.76, 1.96
			C256K (2)	1.96
Pretreatment	T38A		A38K/E (3,5)	2.94, 4.90
	Y124H		H124Y/N/D (36,2,3)	35.29, 1.96, 2.94
	D134E		D134N (1)	0.98
	N139K/H, I224V		N139T (2)	1.96
	R242A		R242A (1)	0.98

a Defined by Liu et al [6], b with defined phenotypic data, c rt139 categories and 3 and 4 were shared, so mutations in a total of 42 positions in the RT region were analyzed. ADV: Adefovir dipivoxil; ETV: Entecavir; Ldt: Telbivudine; LMV: Lamivudine; TNF: Tenofovir

out of the 26 putative positions associated with NAr in the HBV RT region, including 10 previously defined ones and 16 new mutations that differ from the previously defined ones. Out of the 6 pre-treatment RT mutation positions previously defined, treatment mutation was identified total 8 mutation in 5 of them. Additionally, 8 mutations were identified in total, including 7 new types that differ from the previously defined ones. The Y54H mutation was the most frequently observed variation. These data indicate a high prevalence of amino acid variations in the Pol/RT region of the HBV/D genotype. Further studies at the phenotypic level are needed, particularly to assess the relationship of the detected variations, including Y54H, with NAs.

The HBV RT region contains seven functional domains (G, F, A, B, C, D, and E) and five intermediate domains associated with these functional domains (F-A, A-B, B-C, C-D, and D-E) [6, 10, 11, 12]. Putative and pre-treatment mutations generally occur more frequently in the RT A-B intermediate domain, which overlaps with the 'a' determinant region of HBsAg. Thus, mutations occurring in the gene regions overlapping with the S gene in the RT region have the potential to alter HBV virulence, replication capacity, immune recognition, and antigenicity. Mutations in this domain, specifically at positions rt124, rt126, rt128, rt134, rt139, and rt153, have been reported as the most frequently detected variations [6, 7, 10, 11, 12]. In this study, the A-B interdomain of HBV RT was identified as the domain with the most frequent mutation variations. The rtD134N mutation, which overlaps with the HBsAg "a" determinant and can lead to the sI126N/S mutation, was identified in this domain. Additionally, mutations were detected at positions rt124, rt126, rt128, and rt139, which are reported as the most frequently observed variations in this domain.

In treatment-naive patients, 27 different polymorphic sites were identified in the HBV RT region. Among these, rt53, rt54, and rt91 are the most frequently genotype-dependent polymorphic amino acid sites across HBV A/B/C/D/E/F genotypes. Additionally, it has been reported that polymorphisms can be characterized by lower DNA levels [6, 8]. It has been indicated that the HBV/D genotype contains higher genetic diversity compared to other genotypes [13]. In this study, 13 out of the 21 amino acid positions with detected mutations were identified as genotype-associated polymorphic sites. And the previously described rt53, rt124, and rt256 were the most frequently polymorphic amino acid positions.

The effects of pre-treatment mutations occurring in the RT region in treatment-naive patients on the development and progression of progressive liver diseases such as HBV-related cirrhosis and hepatocellular carcinoma (HCC) have not yet been clearly defined. However, mutations such as rtL80I, rtD134N, rtN139K/T/H, rtY141F, rtM204I/V, rtF221Y, rtI224V, and rtM309K are suggested to be associated with the development of cirrhosis and HCC. [14-17]. In this study, mutations reported to be associated with progressive liver diseases such as cirrhosis and HCC, namely rtD134N in one patient, rtN139T in two patients, rtF221Y in eight patients, and rtM309K in eight patients, were detected. However, in this study, no statistically significant difference was observed between CHB patients with and without NAr mutations in terms of liver enzymes (AST, ALT, AFP) and liver damage-related data, including necroinflammatory activity and fibrosis stages (Tablo 2).

In some studies, the presence of pre-treatment Reverse Transcriptase (RT) mutations has been reported to be associated with lower Hepatitis B Virus (HBV) DNA levels in individuals with HBeAg-negative serostatus. Variations in the NAr-associated HBV RT region are suggested to impair viral replication by reducing polymerase enzyme activity [18, 19]. In this study, no statistically significant difference was observed in virological features, including HBV DNA levels, between CHB patients with detected and undetected NAr mutations. (Tablo 2).

The presence and prevalence of pre-treatment HBV RT mutations in CHB patients can be associated with epidemiological factors, HBV genotypes, viral replication dynamics, HBeAg serostatus, mutation detection methods, the presence of liver damage, and coexisting co-infections. However, the impact of pre-treatment HBV RT mutations on the progression of HBV-associated disease with antiviral treatment in CHB patients is not yet clear. The data obtained from this study indicate a high prevalence of amino acid variations in the HBV RT region in patients with HBV/D genotype. However, it suggests limited effects on antiviral treatment and the progression of HBV-associated disease. Along with this also, the detection of mutations related to potential antiviral drug resistance in treatment-naive patients with chronic B hepatitis can contribute to the development of more effective treatment strategies and clinical management.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Trépo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet. 2014;384(9959):2053-2063.

2. Sonderup MW, Spearman WC. Global disparities in hepatitis B elimination-a Focus on Africa. Viruses. 2022;14(1):82.

3. Summers J, Mason WS. Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. Cell. 1982;29(2):403-15.

4. Lok AS, McMahon BJ, Brown RS Jr, Wong JB, Ahmed AT, Farah F. et al. Antiviral therapy for chronic hepatitis B viral infection in adults: a systematic review and meta-analysis. Hepatology. 2016;63(1):284-306.

5. Caligiuri P, Cerruti R, Icardi G, Bruzzone B. Overview of hepatitis B virus mutations and their implications in the management of infection. World J Gastroenterol. 2016;22(1):145-154.

6. Liu BM, Li T, Xu J, Li XG, Dong JP, Yan P, et al. Characterization of potential antiviral resistance mutations in hepatitis B virus reverse transcriptase sequences in treatment naïve Chinese patients. Antiviral Res. 2010;85(3):512-519.

7. Choi Y-M, Lee S-Y, Kim B-J. Naturally occurring hepatitis B virus reverse transcriptase mutations related to potential antiviral drug resistance and liver disease progression. World J Gastroenterol. 2018;24(16):1708-1724.

8. Mirandola S, Sebastiani G, Rossi C, Velo E, Erne EM, Vario A et al. Genotype-specific mutations in the polymerase gene of hepatitis B virüs potentially associated with resistance to oral antiviral therapy. Antiviral Research. 2012;96(3):422-429.

9. Zhang Q, Liao Y, Cai B, Li Y, Li L, Zhang J, et al. Incidence of natural resistance mutations in naïve chronic hepatitis B patients: a systematic review and meta-analysis. J Gastroenterol Hepatol. 2015;30(2):252-261.

10. Banerjee P, Chakraborty A, Mondal RK, Khatun M, Datta S, Das K. HBV quasispecies composition in Lamivudine-failed chronic hepatitis B patients and its influence on virological response to Tenofovir based rescue therapy. Sci Rep. 2017;17(7):44742.
11. Zheng J, Zeng Z, Zhang D, Yu Y, Wang F, Pan CQ. Prevalence and significance of Hepatitis B reverse transcriptase mutants in different disease stages of untreated patients. Liver Int. 2012;32(10):1535-1542.
12. Yamani LN, Yano Y, Utsumi T, Wasityastuti W, Rinonce HT, Widasari DI, et al. Profile of mutations in the reverse transcriptase and overlapping surface genes of hepatitis B virus (HBV) in treatment-naïve indonesian HBV carriers. Jpn J Infect Dis. 2017;70(6):647-655.
13. Chu CJ, Keeffe EB, Han SH, Perrillo RP, Min AD, Soldevila-Pico C, et al. Prevalence of HBV precore/core promoter variants in the United States. Hepatology 2003;38(3):619-628.
14. Kim JE, Lee SY, Kim H, Kim KJ, Choe WH, Kim BJ. Naturally occurring mutations in the reverse transcriptase region of hepatitis B virus polymerase from treatment-naïve Korean patients infected with genotype C2. World J Gastroenterol. 2017;23(23) 4222-4232.
15. Huang CJ, Wu CF, Lan CY, Sung FY, Lin CL, Liu CJ et al. Impact of genetic heterogeneity in polymerase of hepatitis B virus on dynamics of viral load and hepatitis B progression. PLoS One. 2013;8(7):e70169.
16. Li H, Jia J, Wang M, Wang H, Gu X, Fang M, et al. F221Y mutation in hepatitis B virus reverse transcriptase is associated with hepatocellular carcinoma prognosis following liver resection. Mol Med Rep. 2017;15(5):3292-3300.
17. Wu Y, Gan Y, Gao F, Zhao Z, Jin Y, Zhu Y et al. Novel natural mutations in the hepatitis B virus reverse transcriptase domain associated with hepatocellular carcinoma. PLoS One. 2014;9(5):e94864.
18. Zhu B, Wang T, Wei X, Zhuo Y, Liu A, Zhang G. Accumulation of mutations in reverse transcriptase of hepatitis B virus is associated with liver disease severity in treatment-naïve Chinese patients with chronic hepatitis B. Adv Clin Exp Med. 2017;26(7):1123-1129.
19. Kirishima T, Okanoue T, Daimon Y, Itoh Y, Nakamura H, Morita A. et al. Detection of YMDD mutant using a novel sensitive method in chronic liver disease type B patients before and during lamivudine treatment. J Hepatol. 2002;37(2):259-265.

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